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Primordial Germ Cells of *Synaptula hydriformis* (Holothuroidea; Echinodermata) Are Epithelial Flagellated-Collar Cells: Their Apical-Basal Polarity Becomes Primary Egg Polarity

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Abstract. The primordial germ cells (PGCs) of a recently metamorphosed juvenile *Synaptula hydriformis* occur with somatic cells in the germinal epithelium of the gonad. As part of the epithelium, PGCs rest on a basal lamina, extend apically towards a lumen, are joined to other cells of the epithelium *via* apicolateral junctions, and express apical-basal polarity. Each PGC has an apical flagellum that is surrounded by a collar of microvilli. The apicolateral junctions of PGCs consist of apical adhering and subapical septate junctions. Hemidesmosomes attach the PGCs to the basal lamina. Although the somatic cells form an incomplete layer over the PGCs, both the PGCs and somatic cells remain exposed to the apical lumen and retain contact with the basal lamina. The peritoneum is the outermost layer of the gonad and faces the perivisceral coelom. The epithelial-cell characteristics expressed by cells of the peritoneum are identical to those of the germinal epithelium. PGCs of *S. hydriformis* are epithelial flagellated-collar cells and express the apical-basal polarity that is typical of epithelial cells. The apical-basal polarity of the oocyte, animal-vegetal axis of full-grown eggs, and anterior-posterior axis of larvae and adults are all in correspondence. Thus the polarity of the germinal epithelium may determine the primary body axis of the next generation.

Introduction

Most eggs have an inherent polarity, expressed as the animal-vegetal axis, that is visible at least by the eccentric nucleus and site of production of polar bodies (Wilson, 1896). Although the relationship of primary egg polarity to embryonic polarity has been scrutinized in a number of species, and the genetic control and expression of both egg and embryo polarity are currently under investigation in a few species, a general model of the origin of egg polarity has not been forthcoming. Thus, although E.L. Mark suggested in 1881 that egg polarity was determined by "the topographical relation of the egg (when still in an indifferent state) to the remaining cells of the maternal tissue from which it is differentiated" (Mark, 1881, p. 515), his hypothesis has never been explicitly tested.

Current research on egg polarity centers primarily on the asymmetric distribution within the egg of maternally derived determinants (see Davidson, 1986, for general information; Gard, 1995, for *Xenopus;* González-Reyes *et al.,* 1995, and Curtis *et al.,* 1995, for *Drosophila*). The initial, spatial relationship of the oocyte to maternal tissues, a possible cause of these asymmetries, has been examined in relatively few species. In *Drosophila melanogaster,* it has been shown that both larval axes (anterior-posterior and dorsal-ventral) are determined by interactions between the oocyte and cells of the maternal egg chamber, resulting in the localization of specific, maternally derived mRNAs (González-Reyes and St. Johnston, 1994; González-Reyes *et al.,* 1995; Curtis *et al.,* 1995). In *Xenopus laevis,* the animal-vegetal axis of the oocyte and egg is determined during oogenesis, and the anterior-posterior embryonic axis "can be roughly superimposed on [it]" (Gard, 1995). Animal-vegetal polarity is hypothesized to be inherent in the oocyte, rather than the result of its spatial relationship to follicular tissue (reviewed in Gerhart *et al.,* 1983; Gard, 1995), but the establishment of dorsality is related to the point of

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sperm entry and rotation of egg contents (Gerhart *et al.*, 1989). Echinoderm eggs also become polarized along the animal-vegetal axis during oogenesis (Boveri, 1901a, b; Jenkinson, 1911; Hörstadius, 1973; Maruyama *et al.*, 1985), and the relationship of the oocyte to maternal tissue is thought to establish animal-vegetal egg polarity (Schroeder, 1980a, b, 1985, 1986; Smiley, 1988).

One obstacle to understanding the relationship between the oocyte and maternal tissues has been the difficulty in identifying a marker of polarity that is present in the oocyte before it is released from the tissue of the ovary (Mark, 1881). "What is needed is to trace the polarity back to its earliest manifestation and to discover some ultrastructural [or other marker]" (Schroeder, 1980a) that is visible while the oocyte-somatic tissue relationship can be documented.

Holothuroid oocytes are visibly polarized cells. The animal pole is identified by a structure termed the apical protuberance (first used by Smiley, 1988) and an apically displaced nucleus. The protuberance is an area of yolk-deficient ooplasm that protrudes from the surface of the oocyte, and is visible using both light microscopy and electron microscopy (Smiley and Cloney, 1985; Smiley, 1988; Frick *et al.*, 1996). Polar bodies are produced at the apical protuberance (Maruyama, 1980; Smiley and Cloney, 1985). Because the protuberance develops while the holothuroid oocyte is still associated with other cells and tissues of the ovary, yet remains visible in the spawned, mature egg as either the protuberance itself (Smiley and Cloney, 1985) or as a defect in the jelly layer (Frick, unpub. data), the polarity of the spawned egg can be related to that of the oocyte within the ovary.

Using the apical protuberance and apically displaced nucleus as markers, we showed that the animal-vegetal polarity of the full-grown oocyte of *Synaptula hydriformis* corresponds with the apical-basal polarity of the parental germinal epithelium (Frick *et al.*, 1996). It has also been reported that the polarity of *Parastichopus californicus* oocytes is identical to epithelial polarization (Smiley, 1988). By tracing morphogenesis from oogonia to full-grown oocytes in *S. hydriformis*, we determined that the polarity of the egg, at all stages of morphogenesis, parallels the polarity of the germinal epithelium. Because gonial cells and previtellogenic oocytes also expressed structures typical of epithelial cells, such as an apical rudimentary flagellum, we hypothesized that the germ cells might be derived from epithelial cells (Frick *et al.*, 1996).

Epithelial cells, in general, rest on a basal lamina and form junctions (hemidesmosomes) with it; they extend apically towards a lumen or space; they form specialized, apicolateral, junctional complexes (among invertebrates, usually a zonula adherens and a septate junction) with neighboring cells; and they express apical-basal polarity, as indicated by, for example, an apical flagellum (Welsch and Storch, 1976; Hay, 1990; Ruppert and Barnes, 1994).

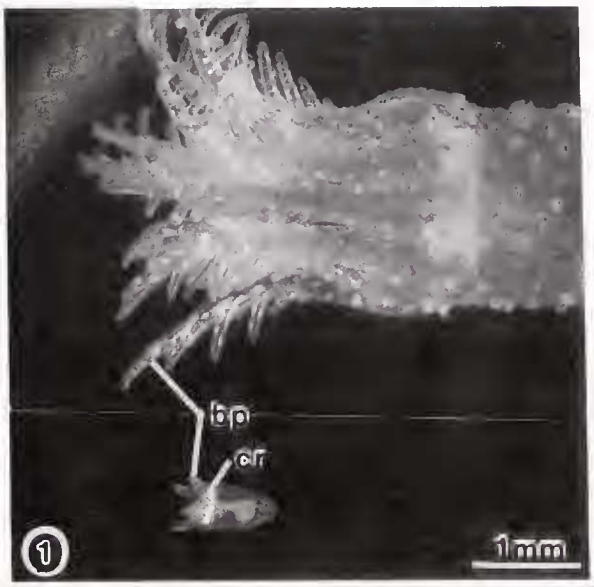
Primordial germ cells (PGCs) are defined by Wilson (1896) as the first germ cells that are "clearly distinguishable from the somatic cells." PGCs are sexually indifferent and capable of migration; gonial cells are sexually determined and nonmigratory (Wilson, 1896; Wourms, 1987). Both PGCs and gonial cells divide by mitosis (Wourms, 1987). According to these definitions, PGCs and gonial cells are differentiated on the basis of genetics and behavior, not structure. In the absence of any structural criteria with which to distinguish between PGCs and gonial cells, we consider the germ cells of juvenile *Synaptula hydriformis* to be PGCs, rather than oogonia, because the juvenile is recently metamorphosed and the gonad is rudimentary. In the adult gonad (Frick *et al.*, 1996), we considered the population of morphologically similar cells that contained dividing cells to be oogonia and spermatogonia.

Nuage, or germ plasm, is considered to be a marker of germ cells (Eddy, 1975). Nuage is known to occur in echinoderm germ cells (Eddy, 1975); in the holothuroid *Parastichopus californicus*, nuage does not appear in the PGCs until 6 months after metamorphosis (Smiley, 1988), but in the echinoid *Lytechinus pictus*, nuage appears in the PGCs of 3-week postmetamorphic urchins (Houk and Hinegardner, 1980). In *Synaptula hydriformis*, nuage has been described from previtellogenic oocytes (Frick *et al.*, 1996). It appears in the perinuclear region of the cytoplasm as one or more clumps of amorphous, electron-dense, non-membrane-bound material that is frequently associated with mitochondria (Frick *et al.*, 1996).

We have undertaken this study of the ovotestis of a recently metamorphosed juvenile of *Synaptula hydriformis* to determine whether PGCs are epithelial cells and if their polarity corresponds to that of the germinal epithelium. If so, we predict that PGCs (in this species destined to become *both* spermatogonia and oogonia) should express the characteristics of typical invertebrate epithelial cells, as noted above. A demonstration that PGCs are epithelial and polarized would imply that both sperms and eggs are, at least primitively, intrinsically polarized cells. In the case of eggs, this polarity would manifest itself as animal-vegetal, primary egg polarity.

Materials and Methods

Adult specimens of *Synaptula hydriformis* were collected in Lake Surprise, Key Largo, Florida, and transported to the Smithsonian Marine Station in Fort Pierce, Florida (described in Frick *et al.*, 1996). Brooded juveniles were removed from the adult perivisceral coe-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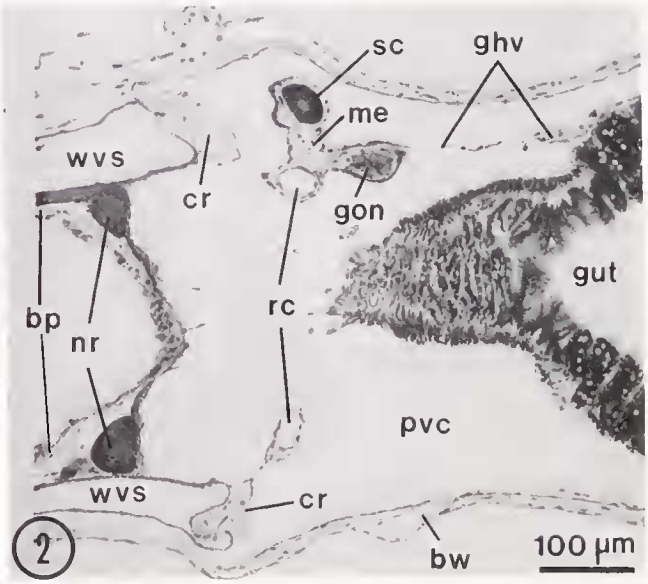
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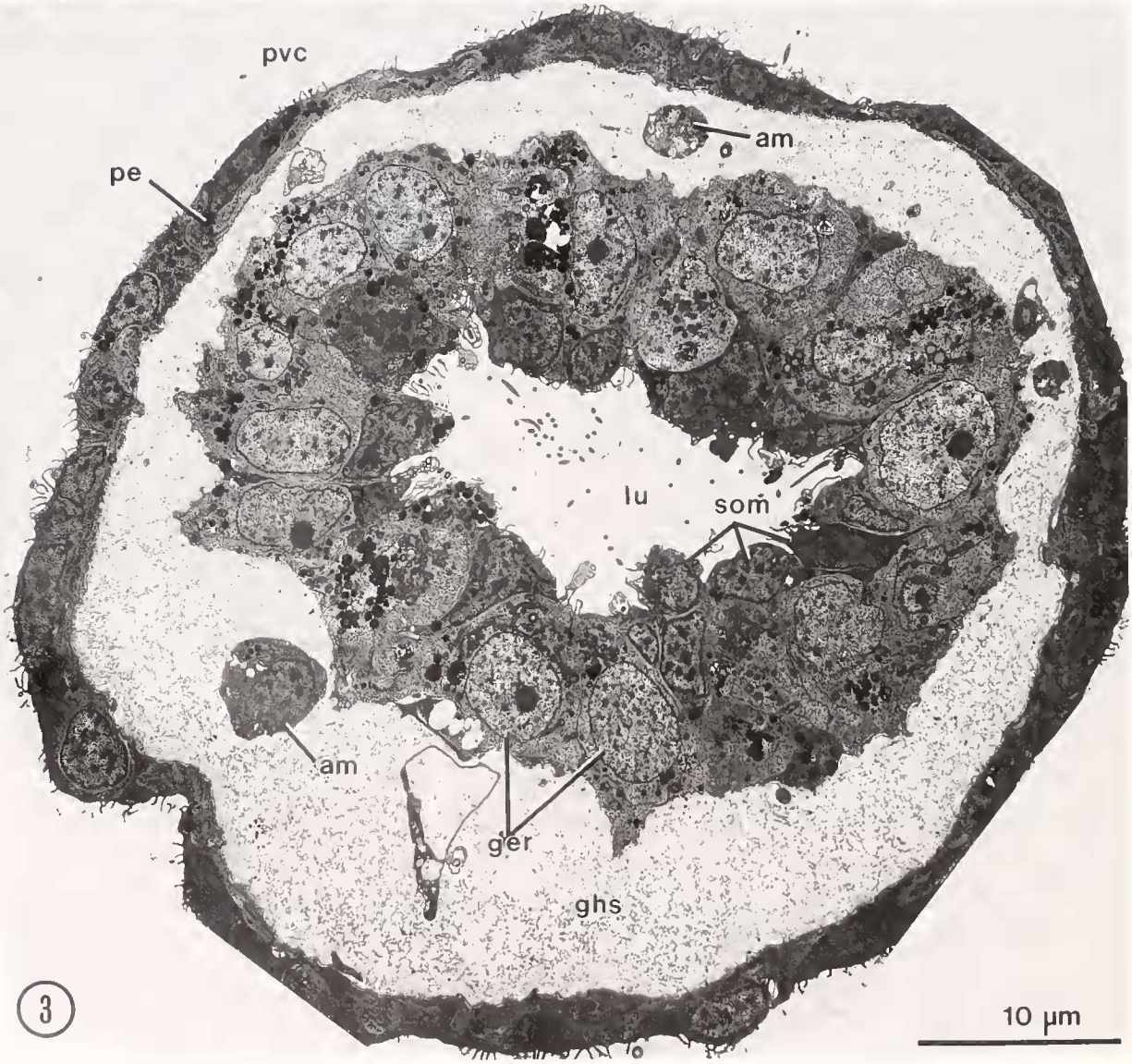
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lom, relaxed in isosmotic $MgCl_2$ mixed equally with seawater, and fixed. Primary fixation was at room temperature in isosmotic 2.5% glutaraldehyde in Millonig's 0.2 *M* phosphate buffer for 1.5 h; secondary fixation was in isosmotic 1% osmium tetroxide in Millonig's 0.1 *M* phosphate buffer for 1 h. Whole juveniles were dehydrated in a graded series of ethanols and embedded in epoxy resin. Before sectioning, the body wall, which contained calcareous ossicles, was trimmed from the block. Sections were cut on an MT2B ultramicrotome. Thick sections for light microscopy were stained with 1% methylene blue and 1% azure II and photographed with a Zeiss photomicroscope III. Thin sections for transmission electron microscopy were stained with 6% alcoholic uranyl acetate and Reynold's lead citrate and were photographed with a Zeiss EM9S2. Living adults and juveniles were photographed with a Nikon macro lens.

Results

Brooded, recently metamorphosed juveniles of *Synaptula hydriformis* have five buccal podia, a calcareous ring, and a gut that are visible externally or through the transparent body wall (Fig. 1). In sectioned animals, ampullae of the buccal podia, other parts of the water-vascular system, the circumesophageal nerve ring, the calcareous ring, and the gut are visible (Fig. 2). The gonad is present in juveniles and is lodged in the dorsal mesentery, as are the stone canal and ring canal of the water-vascular system (Fig. 2). A hemal sinus surrounds the gonad and is joined to the hemal system of the gut by a hemal vessel that passes through the mesentery (Fig. 2).

The juvenile gonad consists of three layers (Fig. 3). An outer peritoneum faces the perivisceral coelom, an inner germinal epithelium surrounds the gonadal lumen, and a connective-tissue layer is situated between the two epithelia.

The connective-tissue layer and the genital-hemal sinus occupy the same tissue compartment, and structural connective tissue grades into hemal fluid. Immediately beneath the peritoneal basal lamina, a few collagen fibers are present, but slightly deeper into the connective tissue, collagen is replaced by the abundant proteins of the hemal fluid (Fig. 3). Cells within the genital-hemal sinus are amebocytes (Fig. 3), as described from the adult gonad (Frick *et al.*, 1996).

The simple germinal epithelium is composed of germinal and somatic cells. The germinal epithelium of adult *Synaptula hydriformis* produces both sperm and eggs (see Frick *et al.*, 1996); thus the PGCs of the juvenile gonad differentiate into oogonia and spermatogonia. The germ cells are more or less cuboidal in shape and make the epithelium a thick, 10–15-μm layer (Fig. 3). The germinal and somatic cells are visibly distinct from one another (Fig. 4).

PGCs have a large, 7-μm circular nucleus with chromatin distributed in sparse clumps and a prominent nucleolus (Figs. 3–8). Their diffuse but uniform cytoplasm stains more lightly than that of the somatic cells (Figs. 3–8). PGCs contain nuage (Figs. 7, 8). Mitochondrial profiles are common throughout the cytoplasm (Figs. 3–8). Golgi bodies are primarily apical in position (Figs. 5–7). Dark, lipoidal inclusions, which are not membrane-bound, are found in both PGCs and somatic cells (Figs. 3–10).

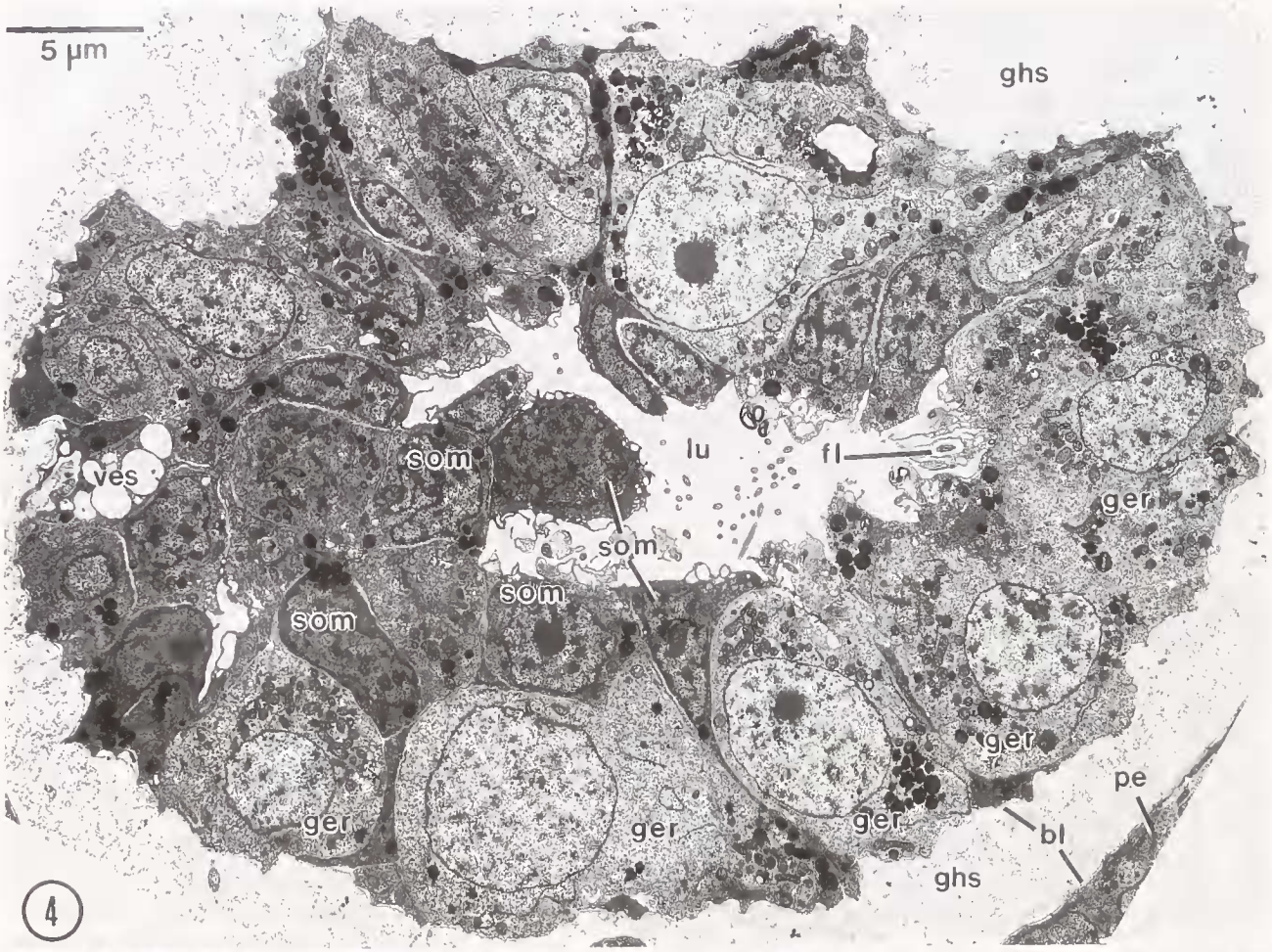
PGCs display characteristics of epithelial cells. They are part of a sheet of cells that rests on a common basal lamina (Figs. 3, 4), they are joined by intercellular junctions to other cells (Figs. 5, 6), and the cells express an apical-basal polarity (Figs. 5, 6). Each PGC has an apical flagellum that extends into the lumen of the tubule (Figs. 5, 6). The flagellum is surrounded by a collar of microvilli (Figs. 5, 6), and a striated rootlet fiber anchors it to the nuclear membrane (Fig. 5). The Golgi bodies noted above are frequently associated with the flagellar base (Figs. 5–7). The apicolateral junctions of PGCs are complex and consist of an apical adhering junction (probably a zonula adherens) and a subapical septate junction (Fig. 6 inset). Hemidesmosomes join the PGCs to the basal lamina on which they rest (Fig. 7 inset).

The perikarya of the somatic cells of the germinal epithelium form an incomplete layer over the PGCs, partially separating them from the apical lumen and the basal lamina (Figs. 3–4, 8). Part of the apical surface of

Figure 1. Adult and juvenile *Synaptula hydriformis* (photomacrograph). The juvenile was removed from the perivisceral coelom of the adult. Buccal podia (bp), calcareous ring (cr).

Figure 2. Longitudinal section of juvenile (light micrograph). The nerve ring (nr), calcareous ring (cr), and ring canal (rc) encircle the esophagus (not in plane of section). The gonad (gon) is embedded in a mesentery (me) that extends from the ring canal (rc) to the gut (gut). Within this same mesentery, a hemal vessel (ghv) and the stone canal (sc) also occur. Buccal podia (bp), body wall (bw), perivisceral coelom (pvc), water vascular system (wvs).

Figure 3. Transverse section of juvenile gonad (transmission electron micrograph). The peritoneum (pe) faces the perivisceral coelom (pvc). The genital-hemal sinus (ghs) contains amebocytes (am) and proteins. The germinal epithelium is composed of both germinal (ger) and somatic (som) cells and faces the gonadal lumen (lu).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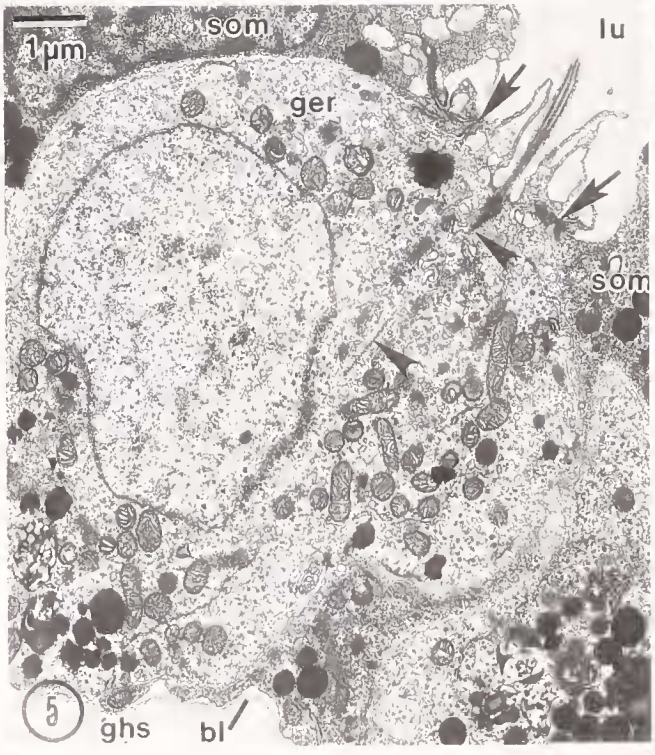
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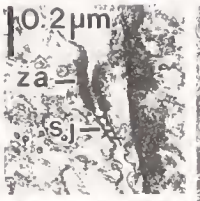
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each PGC, however, is exposed to the lumen, and part of the basal surface rests on the basal lamina (Figs. 4–6). The perikaryon of the somatic cell is displaced apically, but the cell remains in contact with the basal lamina *via* slender cellular processes that pass between the germ cells (Figs. 4, 7, 8). Thus, the germinal epithelium is a simple, but pseudostratified, epithelium composed of germ and somatic cells. The nuclei of somatic cells are smaller than those of germinal cells (Fig. 4). They are irregularly shaped and contain discrete patches of heterochromatin, much of which is peripherally located (Figs. 3–8). They may also possess a nucleolus (Figs. 3, 4). The dense cytoplasm of somatic cells stains more darkly than that of germ cells (Fig. 3–8). Although some mitochondria are present (Figs. 4, 6), few other structures except lipoidal granules are apparent (Figs. 4–8).

The peritoneum of the gonadal tubule is a flattened epithelium, *ca.* 2-μm thick (Figs. 3, 9–11), that rests on a basal lamina and faces the perivisceral coelom. Nuclei of these somatic epithelial cells are flattened in the plane of the epithelium and contain patches of peripheral heterochromatin (Figs. 3, 9). The cytoplasm, which is moderately dense, contains numerous mitochondria, cisternae of rough endoplasmic reticulum, and lipoidal granules (Figs. 9–11). An apical flagellum, originating from a basal body anchored with a rootlet fiber, extends into the perivisceral coelom and is surrounded by a collar of microvilli (Fig. 9). Microvilli also occur elsewhere on the apical surface of the epithelial cells (Figs. 9, 10). Hemidesmosomes attach the epithelial cells to the basal lamina (Fig. 10). Both longitudinal (Fig. 10) and circular (Fig. 11) muscle fibers, as well as nerves (Figs. 9, 11), occur in the peritoneum. Cells of the peritoneum are joined in a junctional complex identical to that of the germinal epithelial cells. An apicolateral adhering junction underlain by a septate junction joins adjacent cells (Fig. 12).

Discussion

PGCs as epithelial cells

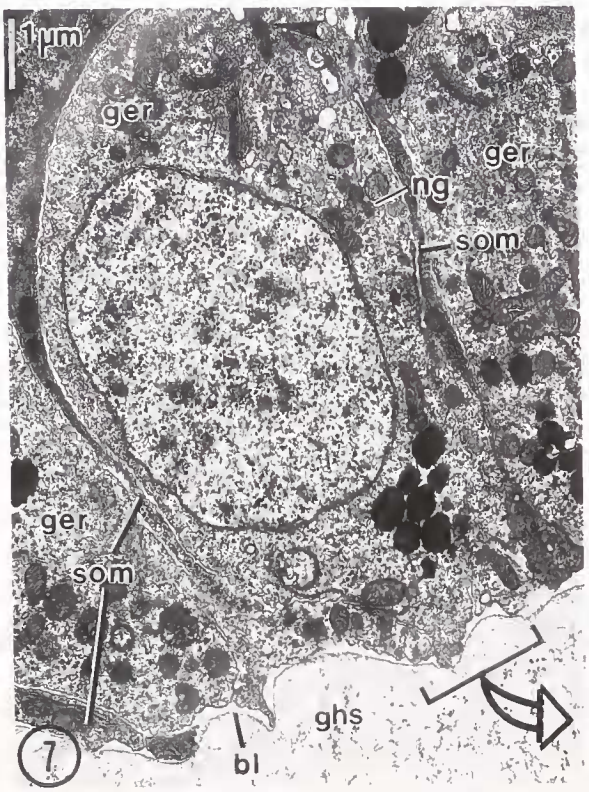
As shown in this study, the PGCs of *Synaptula hydriformis* express all the characteristics of typical invertebrate epithelial cells. These characters are identical to those expressed by epithelial cells of the perivisceral peritoneum, leaving little doubt that the PGCs are epithelial cells. This is the first complete demonstration of the epithelial nature of echinoderm PGCs.

Two other electron-microscopic descriptions of echinoderm PGCs identify some, but not all, epithelial characteristics. In the holothuroid *Parastichopus californicus* (Smiley, 1988), PGCs, oogonia, and previtellogenic oocytes are "attached to the . . . basal lamina," although no hemidesmosomes are reported, and they extend towards the lumen of the tubule. The cells are bound to other cells with intercellular junctions, described as zonulae adhaerentes. PGCs have apical centrioles and "appear to bear a cilium"; a single previtellogenic oocyte was shown to have an apical centriole (Smiley, 1988). Smiley (1988) recognized that these oocyte characteristics were specific to epithelial cells. Our report confirms and expands on his observations. Junctions with neighboring cells, association with basal lamina, and an apical flagellum are variously noted in the previtellogenic or early vitellogenic oocytes of several other species of holothuroids (Eckelbarger and Young, 1992; Tyler *et al.*, 1994; Frick *et al.*, 1996).

The PGCs of echinoids also express some characteristics of epithelial cells. Houk and Hinegardner (1980) reported intercellular "tight junctions" between somatic cells and PGCs and "junctional complexes" between PGCs. They also noted "striated rootlets and flagellar bases" that, though regionally unspecified, are presumably apical in position. The PGCs and oogonia rest on a basal lamina along with other cells of the epithelium (Houk and Hinegardner, 1980; Frick and Ruppert, unpub. data). Furthermore, on the basis of their similar morphology, the PGCs and somatic accessory cells are thought to be derived from a common embryological origin. Both PGCs and somatic cells contain "membrane-bound vesicles filled with an electron-dense material which resembles yolk" (Houk and Hinegardner, 1980), perhaps similar to the lipoidal inclusions seen in *Synaptula hydriformis* PGCs and somatic cells.

The expression of a collared flagellum in the PGCs of *Synaptula hydriformis* (Figs. 5, 6) is significant for two reasons. First, it indicates that the PGC, perhaps after a migratory period, differentiates initially into a specialized cell, *viz.*, an epithelial flagellated-collar cell. Second,

Figures 4–6. Transverse sections of the germinal epithelium (transmission electron micrograph). Fig. 4: Germinal (ger) and somatic (som) cells of the germinal epithelium rest on the basal lamina (bl) and extend into the gonadal lumen (lu). Flagellum (fl), genital-hemal sinus (ghs), peritoneum (pe), vesiculated somatic cell (ves). Figs. 5–6: The base of germinal cells (ger) rests on a basal lamina (bl) and the apex, with a flagellum surrounded by microvilli, extends to the lumen (lu). The cells have apicolateral junctions (arrows) with other cells of the epithelium. The rootlet of the flagellum (arrowheads) is anchored to the nuclear membrane. Inset: The junctional complex between a germinal and somatic cell consists of an apicolateral zonula adherens (za) and a septate junction (sj) just basal to it.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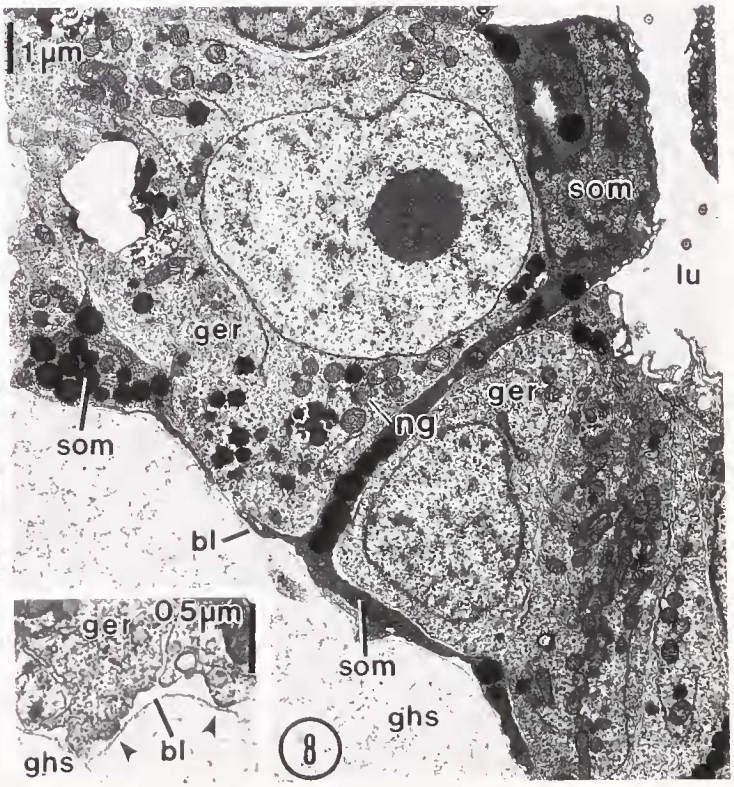
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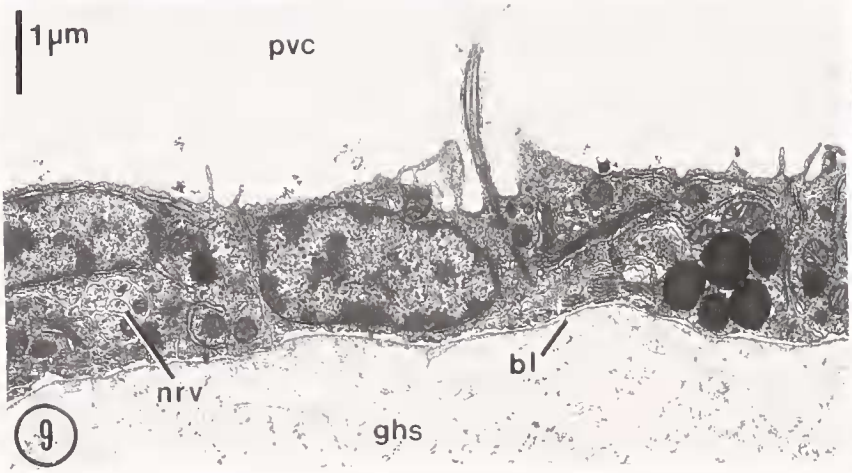
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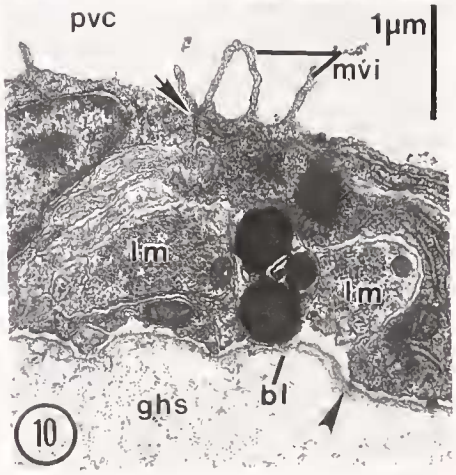
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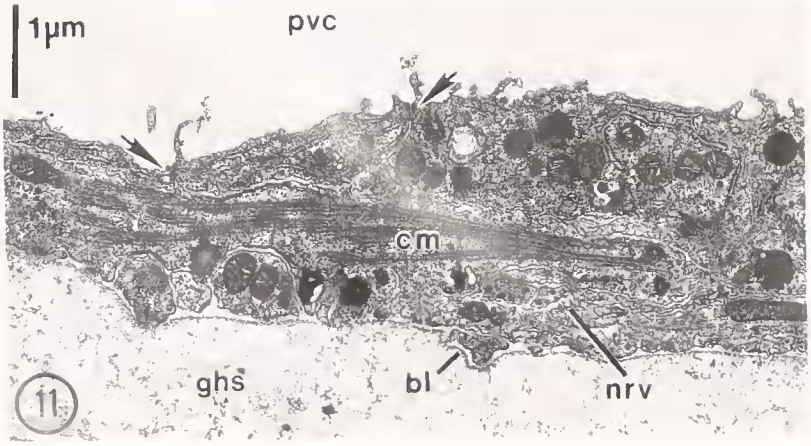
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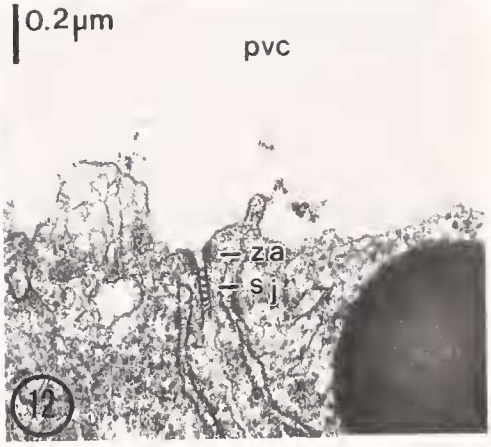
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because the full-grown eggs lack a flagellum, oogenesis is a partial departure, or dedifferentiation, from a differentiated state. The departure is only partial, however, because the egg retains its epithelial polarity as its animal-vegetal axis despite the loss of flagellum, junctions, and shape of a typical epithelial cell (Frick *et al.*, 1996). Thus, the PGCs of *S. hydriformis* are not a reserve of undifferentiated cells but somatically differentiated cells with the capacity to become germ cells.

On the basis of their position within the dorsal mesentery and their morphology, echinoderm PGCs are claimed by some to be mesenchymal (Hamann, 1888; Delavault, 1966; Houk and Hinegardner, 1980; Smiley, 1988), while others classify them as epithelial (Russo, 1902; MacBride, 1936; Nieuwkoop and Sutasurya, 1981; Holland, 1991; Hendler, 1991). This report does not identify the origin of presumptive PGCs in the development of *S. hydriformis*, but does indicate that at the time the PGCs are lodged in the gonad they are epithelial cells; this observation is substantiated by evidence from other holothuroids (Smiley, 1988) and echinoids (Houk and Hinegardner, 1980). Although Houk and Hinegardner (1980) classify echinoid PGCs as mesenchymal, their report shows that the PGCs rest on a basal lamina, form junctions with other cells, and possess components of a flagellum. These are all characteristics of epithelial, not mesenchymal, cells. Similarly, Smiley (1988) describes epithelial characteristics for holothuroid PGCs as discussed earlier, but identifies presumptive PGCs as mesenchymal cells because they are lodged initially in connective tissue. Because epithelial-mesenchymal transitions occur among somatic cells (Welsch and Storch, 1976; Hay, 1990), the possibility remains that the germ cells segregate initially as mesenchymal cells but transform into epithelial cells after incorporation into the gonad.

Origin of egg polarity

We have demonstrated that egg polarity in *Synaptula hydriformis* parallels the polarity of the maternal germinal epithelium (as has Smiley, 1988, for *Parastichopus californicus*). The cells of the epithelium, whether destined to form germ cells or somatic cells, are epithelial cells with an apical-basal polarity. We propose that egg animal-vegetal polarity, therefore, is ultimately derived from the apical-basal polarity of the maternal epithelium. We have not experimentally tested this hypothesis by manipulating developing PGCs or oocytes, but we have demonstrated that animal-vegetal egg polarity in *S. hydriformis* can be traced from the PGC (data herein) to the mature oocyte (Frick *et al.*, 1996) and that it corresponds to apical-basal epithelial polarity at all stages.

Echinoderm eggs are polarized cells, and this primary polarity is established during oogenesis. In several species of echinoids, Schroeder used the jelly canal, polar-body extrusion, and pigment patterns of the egg to identify the primary egg axis (Schroeder, 1980a, b). He hypothesized that the relationship of the egg to maternal tissues caused polarity (Schroeder, 1980a). The apical jelly canal, a channel through the jelly coat (Schroeder, 1980b), is the earliest marker of polarity to appear, and it may be derived from an association with maternal tissue (Schroeder, 1980a, b). Similarly, asteroid oocytes also express an animal-vegetal polarity that can be identified by apical centrioles (Schroeder, 1985; Kato *et al.*, 1990; Schroeder and Otto, 1991), an apically displaced nucleus, an absence of large vacuoles and actin-filled spikes at the animal pole, and a local mechanical weakness at the animal pole (Schroeder, 1985). Holothuroid eggs, as already discussed, express animal-vegetal polarity based on the apical protuberance, apically displaced nucleus (Smiley and Cloney, 1985; Smiley, 1988; Frick *et al.*, 1996), and apical centriole (Smiley, 1988) or flagellum (Frick *et al.*, 1996).

The animal-vegetal polarity of echinoderm eggs and embryos corresponds generally to larval anterior-posterior polarity. This correspondence has been best documented in echinoids by using techniques that include morphological observation, experimental manipulation, and molecular genetic analysis. Boveri (1901b) described

Figures 7–8. Germinal (ger) and somatic (som) cells of the germinal epithelium (transmission electron micrograph). Genital-hemal sinus (ghs), nuage (ng). Fig. 7: Both germinal and somatic cells make contact with the basal lamina (bl). Flagellum (arrowhead). Inset: Germinal cells form hemidesmosomes (small arrowheads) where they contact basal lamina (bl). Fig. 8: Somatic-cell nuclei may partially separate germinal cells from the gonadal lumen (lu), and extensions of the somatic cells may extend to the basal lamina (bl) and partially undercut germinal cells.

Figures 9–12. Cells of the peritoneum (transmission electron micrograph). Fig. 9: Flagellated epithelial cells and nerves (nrv) occur in the peritoneum. The cells rest on a basal lamina (bl) that separates them basally from the genital-hemal sinus (ghs). Apically, they extend into the perivisceral coelom (pvc). Fig. 10: Longitudinal muscle fibers (lm) are present in the epithelial cells and microvilli (mvi) occur apically. Hemidesmosomes (arrowhead) connect cells to the basal lamina and apicolateral junctional complexes (arrow) connect cells to one another. Fig. 11: Circular muscle fibers (cm) are present in epithelial cells. Fig. 12: The apicolateral junctional complexes are composed of a zonula adherens (za) and septate junction (sj).

the position of a circumferential pigment band present in the oocytes, eggs, embryos, and larvae of *Paracentrotus lividus*. Relative to the apical jelly canal (micropyle), the pigment band is subequatorial, in the vegetal half of the egg. The pigment band remains vegetally located throughout embryonic development and eventually moves in through the blastopore, at the posterior end of the larva (Boveri, 1901b). The vegetal pole of the egg thus becomes the posterior end of the larva. Boveri's results (1901a, b) are supported by the experimental manipulations of Hörstadius (reviewed in 1973) and confirmed by Schroeder's (1980a) observations. A molecular genetic analysis of the establishment of the oral axis in echinoid larvae (Cameron *et al.*, 1989) also confirms that the animal-vegetal axis remains fixed from egg to larva and that the oral axis develops with a specific relationship to the animal-vegetal axis. Thus, the animal-vegetal axis of the echinoderm egg gives rise to the animal-vegetal axis of the embryo, which develops into the anterior-posterior axis of the larva and is itself derived from the apical-basal polarity of the maternal epithelium. The polarity of the maternal epithelium, therefore, corresponds to that of the larva.

A feature of larval polarity unique to holothuroids is that the larval anterior-posterior axis is identical to the adult anterior-posterior axis (Smiley, 1986). In other classes of echinoderms, the adult axis is shifted from that of the larval axis (Smiley, 1986). Thus in holothuroids, which embody plesiomorphic echinoderm developmental characteristics (Smiley, 1986), the adult anterior-posterior polarity is the same as the apical-basal polarity of the germinal epithelium from which the egg arose.

The hypothesis that egg polarity is derived from the polarity of the maternal germinal epithelium, whether a simple epithelium or complex ovary, is neither new nor restricted to echinoderms. It apparently originated with Mark (1881; also Wilson, 1896) and embraced oogenesis in all animals. Mark (1881, p. 515) stated: "If, in cases where the egg is directly developed from epithelial cells," the position of "the germinal vesicle bears a constant relation to the free surface of the epithelium from which the egg takes its origin," then "it would be fair to infer the existence of corresponding, though obscured, relations in those cases where . . . the origin of the ovum is less directly traceable to an epithelial surface."

Although the oocytes of all animals may not derive their animal-vegetal polarity from apical-basal epithelial polarity, the correlation may be widespread and phylogenetically ancient. On the basis of data presented here for echinoderms and elsewhere for cephalochordates and cnidarians (Frick *et al.*, 1996), we advance two hypotheses. The first is that the earliest metazoans were composed of polarized, flagellated cells, some of which differentiated as germ cells and retained their polarity. The second is that the determinants of primary egg polarity may be related to the factors that establish the polarity of epithelial cells.

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